Preanalytical Variables in Blood Collection: Impact on Precision Medicine

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Liquid Biopsy Summit
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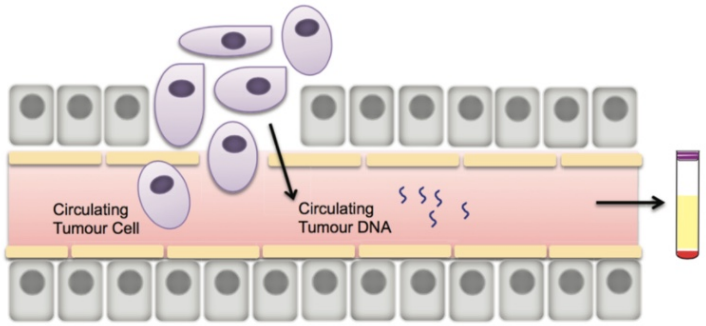
Precision Medicine and Blood-Based Biomarkers

- **Monitoring of health with early detection of disease states**
- **Diagnosis and prognosis of disease based on molecular characteristics**
- **Rational treatment of disease based on specific molecular aberrations**
- **Monitoring of treatment efficacy, toxicity**

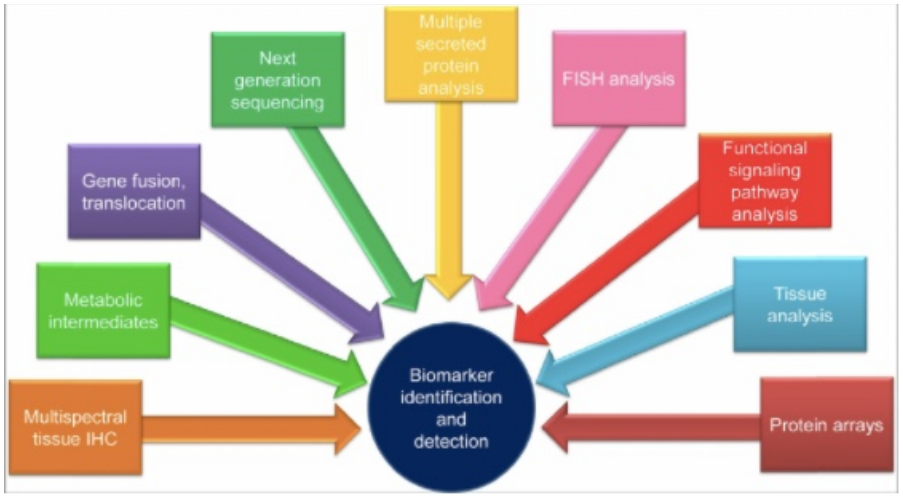
Precision Medicine and Blood-Based Biomarkers

- **Blood is the biospecimen of choice for all of these goals for biomarkers:**
  - **Ready accessibility**
  - **Availability for repeat sampling**
  - **Unique nature of blood as a “systemic” biospecimen**
- **Long been the primary analyte of the clinical pathology laboratory**
  - **Analyses of circulating molecules (proteins, lipids, hormones, electrolytes)**
  - **Analyses of circulating cells (normal, neoplastic)**
- **“Liquid biopsy” is a rebranded blood analysis**
  - **Cell-free DNA**
  - **Circulating neoplastic cells**



What Is a Biomarker?

- **Biomarker: a measurable characteristic used as an indicator of a biological state or condition**
- **Usually a single molecule**
- **Now technologically possible to measure sets of biomolecules across a single class (proteomics profile) or a mixed collection of biomolecules (exosome or CTC contents)**
- **Algorithms are used to define informative patterns**

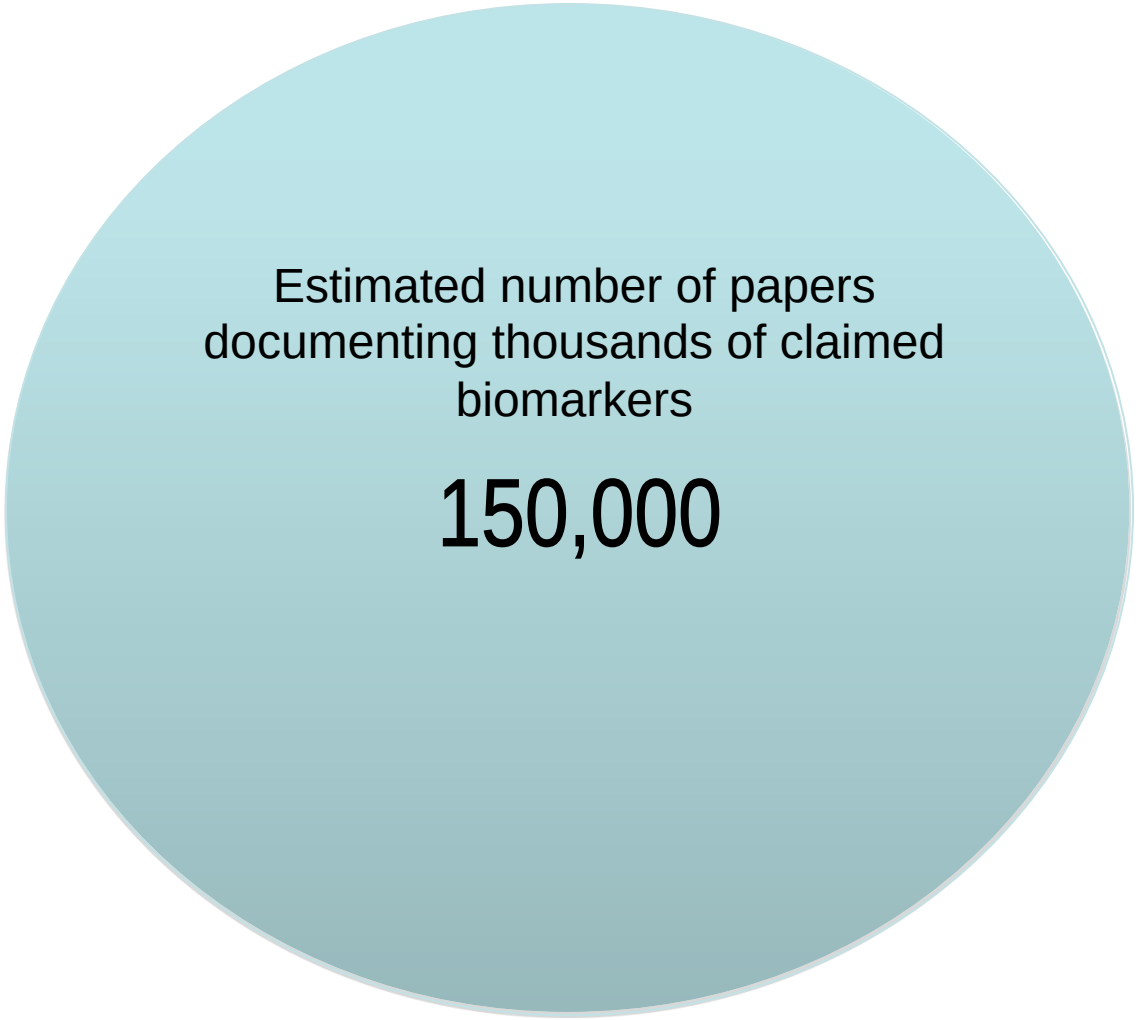


The Biomarker Context of Use (FDA Term) Changes the "Risk": Implications for Decision-Making and Down-Stream Actions

RISK

- **Prognosis**
- **Choice of treatment**
- **Monitoring of treatment**
- **Drug development and clinical trials**
  - **Patient selection**
  - **Toxicity**
  - **Efficacy**
  - **Surrogate endpoints**
- **Early detection, surveillance**
- **None yet used routinely or recommended by USPSTF for screening**

Biomarker Development is Fraught with Failure: Many Putative Biomarkers Are Reported, Few Are Qualified



100

Estimated number of biomarkers routinely used in the clinic

Source: Poste G. Nature 469, 156-157 13 Jan 2011

Bias: A Major Pitfall in Biomarker Validation

JOURNAL OF CLINICAL ONCOLOGY

Official Journal of the American Society of Clinical Oncology

Sources of Bias in Molecular Marker Research in Cancer
- David F. Ransohoff and Margaret L. Gourlay, 2010

Table 1. Sources and "Locations" of Bias in Marker Research

Source of Bias	Location of Bias: Before or After Specimens Are Received in the Laboratory: Before	After	Example
Features of subjects, determined in selection: Age Sex Comorbid conditions Medications	X		Cancer subjects are male, whereas control subjects are mainly female. Bias: Assay results may depend on sex.
Specimen collection	X		Cancer specimens come from one clinic, whereas controls come from a different clinic. Bias: Assay results may depend on conditions that differ between clinics.
Specimen storage and handling	X	X	Cancer specimens are stored for 10 years because it takes longer to collect them, whereas control specimens are collected and stored over 1 year. Bias: Assay results may vary with duration of storage, or with different numbers of thaw-freeze cycles.
Specimen analysis		X	Cancer specimens are run on one day, whereas control specimens are run on a different day. Bias: Assay results may depend on day of analysis in a machine that "wanders" over time.

NOTE. The table shows examples of different sources of bias and the location of the bias before or after specimens are received in the laboratory. The list is not exhaustive; other biases may be important, and the biases listed may or may not be important in any given research study, depending on details of biology and technology (ie, what is being measured and how it might be influenced).

Rigor and Reproducibility: How Is It Assured for Biomarker Measurement in the Clinical Pathology Laboratory?

- **Place** **where test is done**
  - **CLIA/CAP laboratory accreditation**

More is known about the quality of beef in the supermarket than is known about the quality of human biospecimens in clinical care or translational research.

  - **SOPs**
  - **Quality management**
- **Patient samples** **to be tested**
  - **WILD WEST**

Biospecimen Quality Drives Both Molecular Medicine and Translational Research

Analys

DETERMINES QUALITY HERE

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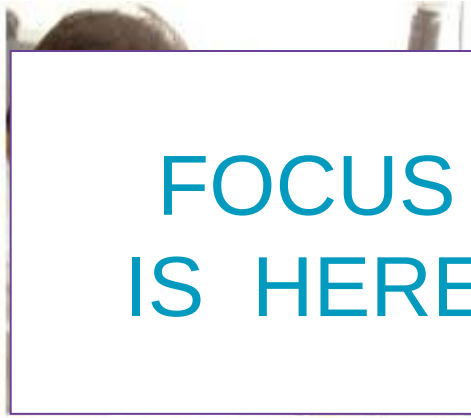
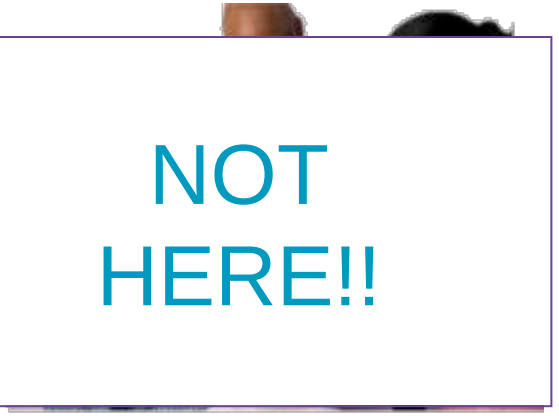


FOCUS IS HERE

PRECISION MEDICINE

NOT HERE!!



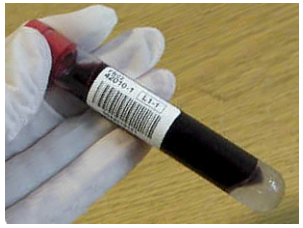
Biospecimen Analysis

Biospecimen Collection

QUALITY HERE

Biospecimen Handling and Processing

Blood Collection and Processing: Preanalytical Variables that Affect Specimen Quality

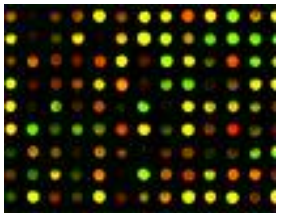


Collection Tubes and Order of Draw

Processing Procedure, Temperature and Time

Distribution & Storage

Molecular Analysis Conditions



Patient Preparation

Blood Draw Procedure

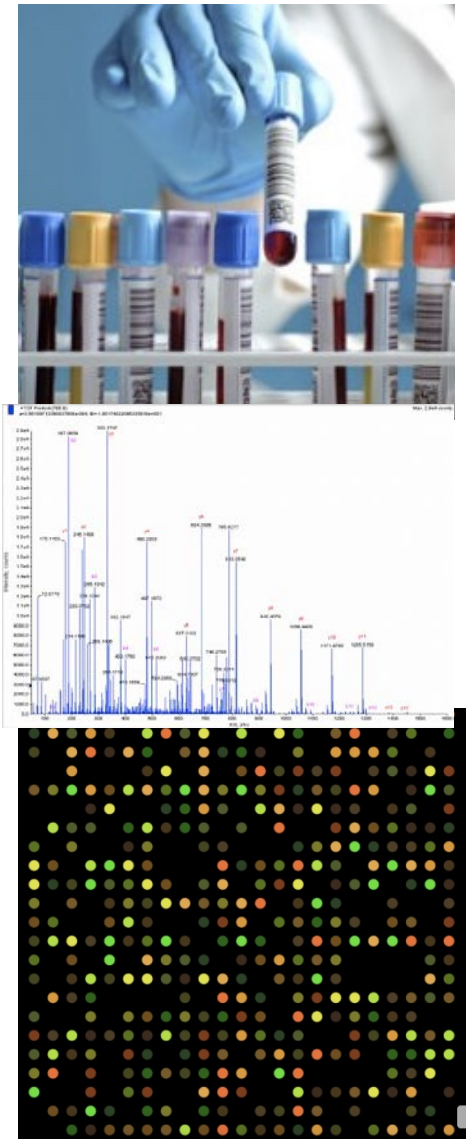
Biospecimen Quality Impacts Both Clinical And Research Outcomes

Effects on Clinical Outcomes

- Potential for incorrect diagnosis
- Potential for incorrect treatment
  - Therapy linked to a diagnostic test

Effects on Research Outcomes

- Irreproducible results
  - Variation in mutation data
  - Variation in gene expression data
- Misinterpretation of artifacts as biomarkers



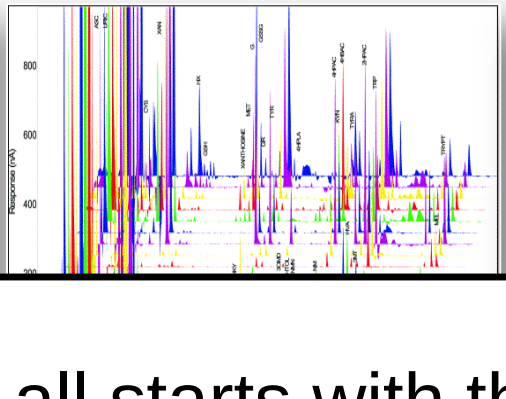
The National Academy of Sciences, Engineering and Medicine (IOM) Speaks

Biomarker Tests for Molecularly Targeted Therapies: Key to Unlocking Precision Medicine

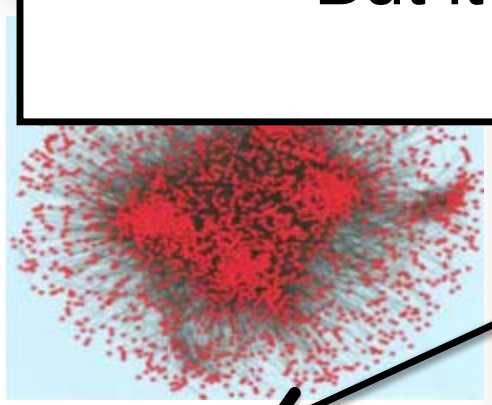
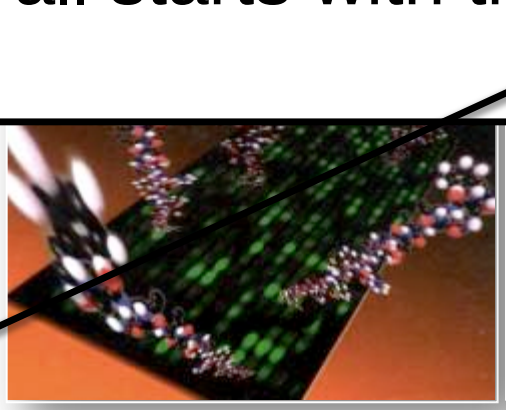
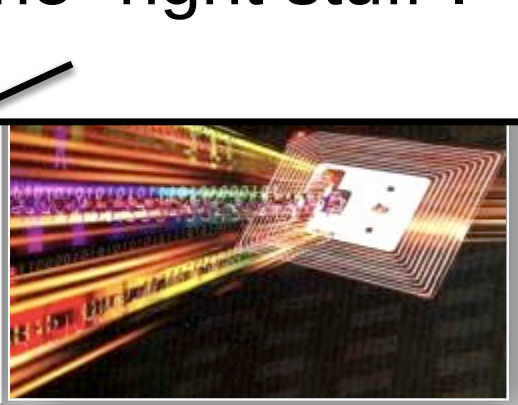
National Academies Press, Jun 30, 2016

- One of the 10 stated goals is to specifically address biospecimen quality.
- Goal 9) Enhance specimen handling and documentation to ensure patient safety and the accuracy of biomarker test results.
  - **“The reliability of biomarker test results depends on the quality of the patient specimens.”**
  - “Professional organizations and health care institutions should develop and implement standards for obtaining adequate specimens.”
    - My current campaign at the College of American Pathologists

And It's Getting Far More Challenging

But it all starts with the "right stuff".


Biospecimens and Analysis of Molecular Pathway/ Network Perturbations

Multiplex Assays and Complex Signal Deconvolution Algorithms

Novel Instrumentation, Automation and Large Scale Informatics

Patient Profiling, Rational Rx and Health Monitoring

Courtesy of G. Poste

Preanalytics and the Liquid Biopsy

- Preanalytical variables of most importance are related to the analyte or analytes that will be assayed and the platform used for the analysis
- Non-cellular elements in plasma
  - Circulating cell-free DNA
  - Circulating cell-free mRNA, miRNA, lncRNA
  - Exosomes (contents: mRNA, miRNA, lncRNA, protein, lipid)
- Circulating tumor cells
  - Enumeration
  - Molecular characterization

Preanalytics and the Liquid Biopsy

- **Most common analyte assayed in liquid biopsies: cell-free tumor DNA**
  - Cancer: detection of mutations
  - Pregnancy: screening for chromosomal disorders

- **Preanalytical variables with (some) data:**
  - Type of specimen (serum vs. plasma)
  - Tube type
  - Specimen handling (time to processing, temperature)
  - Specimen processing (plasma preparation)
  - Storage parameters (temperature, duration)
  - Transport

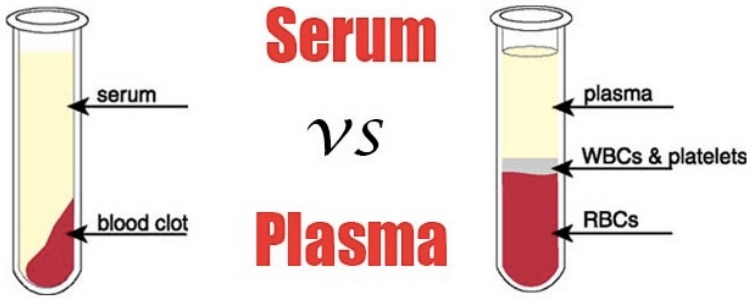
Preanalytics and Cell-Free Tumor DNA

Serum

- Fluid w/o clotting factors
- Acquisition:
  - Allow blood to clot
  - Centrifuge and isolate fluid
- Isolation from blood time consuming and more difficult
- **Contains cell-free DNA**

Plasma

- Fluid with clotting factors
- Acquisition:
  - Centrifuge and isolate fluid
  - Requires an anticoagulant
- Isolation from blood easier and quicker
- **Contains cell-free DNA**



Serum = Plasma – Clotting Factors

Preanalytics and Cell-Free DNA

- **Plasma: The specimen of choice for cell-free DNA testing**
  - Pre-analytic issue: contamination of the circulating tumor DNA with normal DNA from blood leukocytes due to cell lysis
  - Plasma is mostly free of this artifact

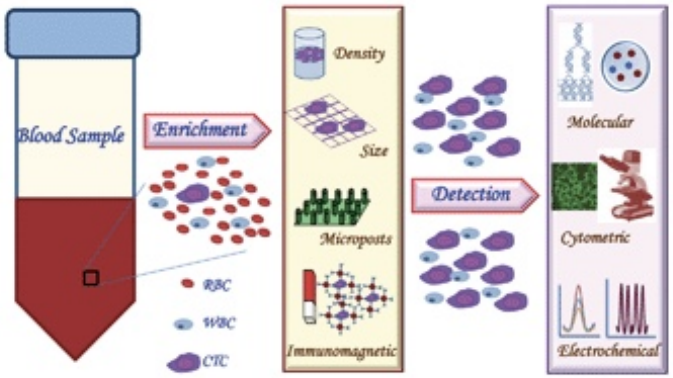
- **But the tube type used affects handling:**
  - Anticoagulant additive: EDTA (lavender-top) tubes
    - Time to processing should be less than 4-6 hours
  - Cell stabilization specialty tubes (several manufacturers)
    - Time to processing more flexible: 48-72 hours
    - Blood stored for 1 week: PAXgene® (QIAGEN) had 10-fold more lysis than BCT® (Streck) in one study
  - All tube types require 10x inversions for mixing

Preanalytics and the Liquid Biopsy

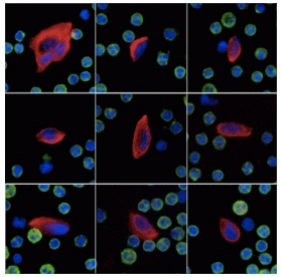
- **Storage**
  - Good news: Plasma extracted at room temperature may be stored frozen prior to DNA extraction with no detrimental effect on DNA quality
- **Transport**
  - Frozen plasma must be transported frozen
  - Cell stabilization tubes: package to minimize temperature fluctuation and sample agitation

Preanalytics and the Liquid Biopsy

- **CTCs**
  - Estimated 10^6 CTCs are shed daily per gram of tumor
  - Short half-life: less than 3 hours in the bloodstream
  - Present in very low concentration amongst millions of blood cells
- **Cytology analysis or molecular analysis?? Either or both!**
  - **Total number** of CTCs and their morphological features
  - **Specific molecular features** of CTCs
    - Protein and nucleic acid content



Preanalytics and the Liquid Biopsy



Circulating tumor cells, typical of those found in cancer patients. Taken from Marrinucci *et al* 2012 *Phys. Biol.* 9

- **Preanalytical issues for CTCs**
  - Isolated from whole blood - must differentiate from normal blood cells
    - Based on physical and/or biological characteristics: size, density, electric charge, deformability, cell surface proteins, viability, membrane properties
  - Morphological and molecular integrity are challenging to preserve during blood sampling and component isolation procedures
  - Tube type: EDTA vs. Citrate vs. specialty preservative-containing tubes
    - EDTA and specialty tubes both preserve CTCs for 48 hours at room temperature
    - Recent data suggests that cell-specific RNA is less well preserved with specialty tubes compared to EDTA tubes (Luk et al. Int J Mol Sci 2017)

Liquid Biopsy Knowledge Gaps

- **Draw variables:**
  - Tourniquet vs. none
  - Tourniquet time
  - Total time of draw
  - Vacuum tube vs. syringe
  - Needle bore
  - Type of port (if used for access)
  - Use or not of discard tube
  - Tube fill level
  - Tube inversions
  - Draw order
  - Draw from central line or artery vs. peripheral vein

Liquid Biopsy Knowledge Gaps

- **Patient variables:**
  - Smoking
  - Exercise
  - Pregnancy
  - Blood pressure
  - Trauma and wound healing
  - Age (age-associated mutations)
  - Pre-malignant / dysplastic disease
  - Non-neoplastic systemic disorders: hematological, immunological, hormonal, inflammatory, cardiovascular, other

Liquid Biopsy Knowledge Gaps

Liquid Biopsy Preanalytics: Proceed with Caution

- Can we really confidently compare results of liquid Bx studies when so many preanalytical variables are unknown and uncontrolled?
- Current state of the science: Focus on analysis and clinical context of use while ignoring many preanalytical issues
- Still early days, but hundreds of clinical trials using liquid biopsies are already in progress and the biomedical literature already contains thousands of publications on liquid biopsies
  - Reproducibility may be challenging
- Urgent need for preanalytical data collection and rigor in techniques extending to the point of care
- More biospecimen research is needed for evidence-based SOPs.

Evidence: as much as needed and no more than necessary …

Preanalytics and Liquid Biopsies

Garbage in…

…Garbage out

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